BEHAVIORAL ECOLOGY

Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*

Erik Thomas Frank,* Thomas Schmitt, Thomas Hovestadt, Oliver Mitesser, Jonas Stiegler, Karl Eduard Linsenmair

Predators of highly defensive prey likely develop cost-reducing adaptations. The ant *Megaponera analis* is a specialized termite predator, solely raiding termites of the subfamily Macrotermitinae (in this study, mostly colonies of *Pseudocanthotermes* sp.) at their foraging sites. The evolutionary arms race between termites and ants led to various defensive mechanisms in termites (for example, a caste specialized in fighting predators). Because *M. analis* incurs high injury/mortality risks when preying on termites, some risk-mitigating adaptations seem likely to have evolved. We show that a unique rescue behavior in *M. analis*, consisting of injured nestmates being carried back to the nest, reduces combat mortality. After a fight, injured ants are carried back by their nestmates; these ants have usually lost an extremity or have termites clinging to them and are able to recover within the nest. Injured ants that are forced experimentally to return without help, die in 32% of the cases. Behavioral experiments show that two compounds, dimethyl disulfide and dimethyl trisulfide, present in the mandibular gland reservoirs, trigger the rescue behavior. A model accounting for this rescue behavior identifies the drivers favoring its evolution and estimates that rescuing enables maintenance of a 28.7% larger colony size. Our results are the first to explore experimentally the adaptive value of this form of rescue behavior focused on injured nestmates in social insects and help us to identify evolutionary drivers responsible for this type of behavior to evolve in animals.

INTRODUCTION

Helping behavior has been observed throughout the animal kingdom, from social insects to primates (*1*). Rescue behavior observed in ants can arise in predator-prey interactions, by rescuing nestmates that have fallen into an antlion trap by digging, pulling the ant out and attacking the antlion, or excavating ants trapped under sand or soil (*2–5*). All hitherto observed types of rescue behavior in social insects were always directed toward individuals under an imminent threat (*1, 6, 7*), that is, suffocation or being eaten. *Megaponera analis* is a strictly termitophagous ponerine ant species, found in sub-Saharan Africa from 25°S to 12°N (*8*) that specializes in raiding termites of the subfamily Macrotermitinae at their foraging sites (*9–13*). A scout ant that has returned to its nest after having found an active termite foraging site initiates a raid. It will recruit approximately 200 to 500 nestmates and lead them to the termites in a column-like march formation, which can be up to 50 m away from the nest (*11, 13, 14*). During the raid, division of labor occurs (*15*): larger ants (majors) break open the protective soil cover created by the termites, whereas the smaller ants (minors) rush into these openings to kill and pull out the prey (*16*). Afterward, the majors collect the dead termites, the column forms again, and the hunting party returns to the nest. These raids occur two to four times a day (*9, 11–13, 17*). Termites have evolved various ways to defend themselves effectively against predators such as *M. analis*, of which a specialized soldier caste with strong sclerotized heads and big mandibles is the main defensive force (*18, 19*). Consequently, ants involved in the hunting process incur high injury risks. We observed a unique helping behavior in *M. analis* to compensate for this high injury rate by carrying back injured ants to the nest. The carrying of ants after the hunt was also observed in Kenya (*13*) and the Democratic Republic of Congo (*20*); however, no attempt was made in those studies to explore the adaptive value of this behavior to the colony or the individual. We further observed the removal of termites, still clinging on to ant extremities in the ant nest, and the rescue behavior toward ants that carry long-term injuries in the form of lost extremities. This specialized rescue behavior is unanticipated in insects, where the value of individuals is generally underestimated, and could provide further proof that empathy is not necessary for helping behavior to emerge in animals (*21*).

RESULTS

Injured *M. analis* ants were antennated by their nestmates at the hunting ground, whereupon they adopted a pupal pose, most likely for ease of transportation back to the nest (movie S1 and fig. S1A). On an average raid, a median of 3 ± 2.9 ants (of 416 ± 153 ants) were carried back ($n = 53$ raids with 154 carried ants), for a total of 9 to 15 rescued ants per day (3 to 5 raids per day). Only in 11% (6 of 53) of the raids were no ants carried back to the nest, and in half of those cases, the raid itself was unsuccessful (no encounter with termites at the hunting ground). If we consider a mean estimated birth rate of 13.3 ± 3.8 ants per day ($n = 5$; for estimate calculation, see the "Quantification of model" section in Materials and Methods), the rescued ants make up a large proportion of the daily turnover in the colony.

Value of rescue behavior for the individual

We classified carried ants into three mutually exclusive categories: (i) ants that partially or completely lost an extremity (antenna or leg), (ii) ants that have termites clinging to their bodies, and (iii) ants that appear to carry no obvious injury (fig. S1B). Most carried ants had a termite clinging on an extremity (Fig. 1A and table S1). This handicap reduces the speed of the ant the most (4.5% of the mean speed of a healthy individual; Fig. 1B and table S1) and, if removed successfully, has no long-term consequences. When 20 randomly selected individuals from each of the three categories of carried ants were forced to return alone from the hunting ground, 32% ($n = 19$ of 60) of them died (Fig. 2A), in contrast to 10% of healthy individuals ($n = 2$ of 20). Ants that were carried back to the nest were never observed to be under any threat of predation ($n = 420$ raids observed during the entire

Department of Animal Ecology and Tropical Biology, Biocentre, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany.
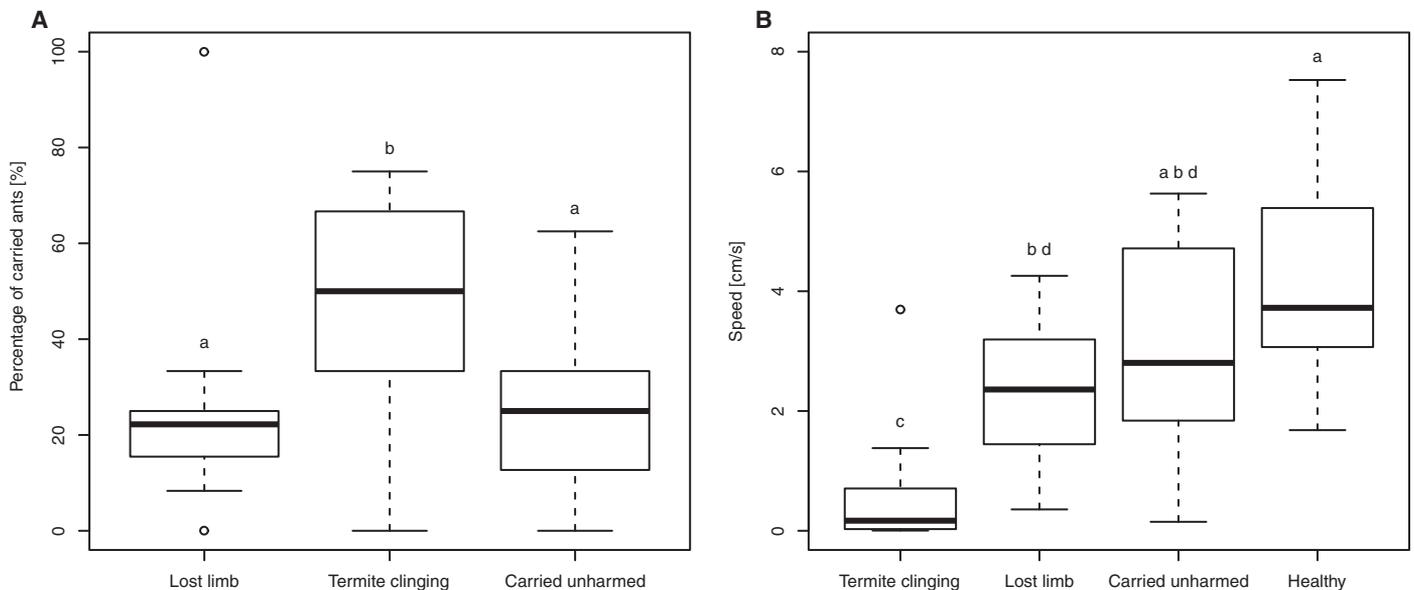*Corresponding author. Email: erik.frank@uni-wuerzburg.de

Fig. 1. Injury-type frequencies and handicap in injured *M. analis* ants. Box-and-whisker plot showing median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers), outliers (>1.5× upper or lower quartile), and significant differences (different letters) for (**A**) distribution of different injury types being carried by helper ants. Lost limb: ant that lost one or more legs or antennas; Termite clinging: ant that still had a termite clinging to its body; Carried unharmed: ant that appears unharmed to the naked eye (Kruskal-Wallis rank sum test, followed by a Dunn's test with Bonferroni correction; *n* = 20 trials with 20 colonies with a total of 154 helped ants). (**B**) Running speed of ants affected by different injuries and healthy individuals as control (Healthy) (Kruskal-Wallis rank sum test, followed by a Dunn's test with Bonferroni correction; *n* = 20 trials with 20 colonies). See also table S1 for detailed statistical results and fig. S1 for illustration of injury types.

field phase), thereby reducing return journey mortality of injured ants from 32% to close to 0%. The main cause of death when ants were forced to return alone was predation by spiders (57.1%: *n* = 12 of the 21 ants killed during the return journey alone from the hunting ground; Fig. 2, B to E). Ants that had a termite clinging on an extremity had the highest mortality rate (50%, *n* = 10 of 20; Fig. 2A). In nature, injured individuals were never observed to return alone without help, but six fatal injuries were observed at the hunting ground (in a total of 53 raids): removed head, thorax, gaster, or multiple legs. These ants were left behind at the hunting ground.

Ants that were carried back to the nest were observed again in subsequent raids 95% of the time (*n* = 38 of 40), sometimes less than an hour after the injury (individuals were marked with acrylic color codes for recognition). Termites clinging onto extremities were removed in 90% of the cases in the following 24 hours without removing the extremity (*n* = 20), thereby completely rehabilitating the handicapped individual. Ants that had lost two randomly selected legs were able to recover in the safe confines of the nest. Twenty-four hours after their injury, they reached mean running speeds 32.1% faster than freshly injured ants, a speed not significantly different from that of healthy individuals (Fig. 3 and table S2).

Of the carried ants, 96.1% were minors (*n* = 154 in 20 observed raids). This is also reflected by the fraction of injured individuals in raiding columns before the fight. A significantly larger fraction of intermediates and minors had lost an extremity compared to majors (Fig. 4 and table S3). The few majors that were carried either had a termite clinging on them or had lost an extremity; they never appeared unharmed.

Focus of rescue behavior
To show that this behavior is focused on injured nestmates, we artificially injured individuals by removing one leg on each side. These in-

dividuals were then placed at the front of the return column, forcing all ants in the column to walk past the injured individual. Whereas healthy and dead individuals were ignored or disposed of by their nestmates, the artificially injured individuals were picked up and carried back to the nest (Fig. 5A, table S4, and movie S1). Artificially injured individuals from other colonies were always attacked and removed from the column (Fig. 5A and table S4). Rescue behavior occurred both directly at the hunting ground and on the return journey, whereas artificially injured ants on the way to the termites were ignored (Fig. 5B and table S4).

Gland and pheromone triggering rescue behavior
When looking for the signal triggering this rescue behavior, we first ruled out stridulation (22), a mechanism known to trigger helping behavior in other ants (1, 7). We observed artificially injured ants, on which stridulation was inhibited, to still be rescued (*P* < 0.001; *n* = 20; fig. S2, A and B, and table S4). After extensive behavioral experiments on dummies (frozen minors), we were able to identify the mandibular gland as the most likely candidate, while ruling out hindgut content and the Dufour's and poison gland reservoirs as triggers of the behavior. To further test this hypothesis, we applied the mandibular gland contents onto healthy individuals; we found that healthy ants covered with mandibular gland material were then carried back by their nestmates (Fig. 5C and table S4). A gas chromatography–mass spectrometry (GC-MS) analysis identified dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS) as the main chemical components of the gland, confirming a previous analysis of the gland contents and concentrations (14 ng of DMDS and 5 ng of DMTS per gland) (10). Although 9 ng of DMDS alone was not enough to trigger the rescue behavior on a dummy, 9 ng of DMTS by itself sufficed. A more pronounced response was achieved with an equal mixture of the two components (9 ng of DMDS and DMTS each) (Fig. 5D and table S4).
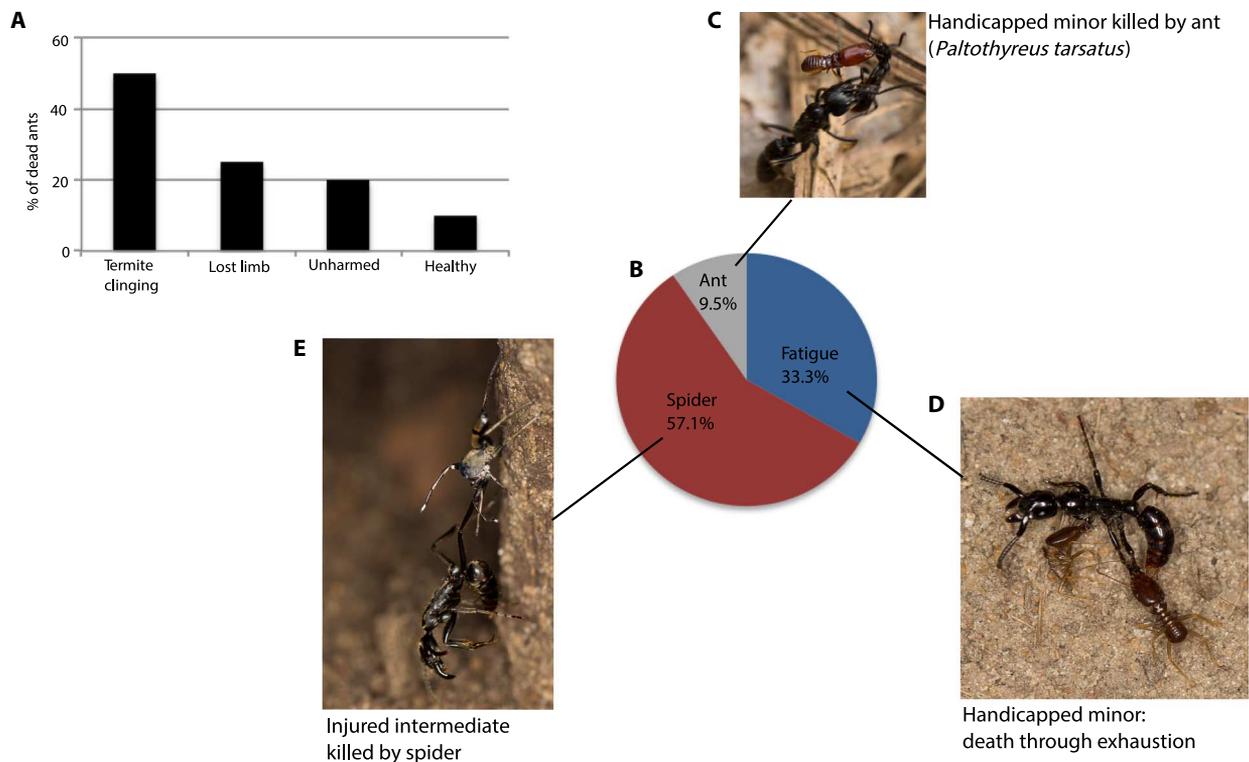
Fig. 2. Mortality and predation of injured and handicapped individuals if not rescued. (**A**) Percentage of injured and handicapped ants dying during the return journey for the three classified carried ant types and control (Termite clinging, Lost limb, Unharmed, and Healthy) if not helped ($n = 20$ for each type, total $n = 80$). (**B**) Percentages of the different mortality causes during the return journey ($n = 21$ of 80 died); spider (red): killed by a predatory spider; fatigue (blue): ant stops moving during return journey, most likely because of exhaustion; ant (gray): injured minor carried off/killed by another ant. (**C**) Handicapped minor with a termite clinging on an extremity carried off by a forager of *Paltothyreus tarsatus*. (**D**) Handicapped minor with two termite soldiers clinging on extremities stops moving because of exhaustion after a 52-min return journey. (**E**) Intermediate with a lost extremity returning alone from the hunting ground ambushed by a Salticidae spider (jumping spider).

Value of rescue behavior for the colony

The rescue behavior in *M. analis* reduces the foraging costs through a reduced mortality risk. We provide a simple analytical model (additional information in "Rescue behavior model" and "Quantification of model" sections in Materials and Methods) that identifies critical factors promoting the evolution of this rescue behavior and why it may have evolved so rarely.

We consider that this behavior could only emerge in species that forage or hunt in groups and in a limited spatial domain so that injured individuals are likely detected by other nestmates. Our model identifies three additional key variables that affect the potential benefit of this rescue behavior: (i) the product of the absolute rate at which ants are severely injured (or killed) in conflict with termites (ε_H) and the fraction f ($0 \leq f \leq 1$) of these ants that could profit from the rescue behavior; (ii) the baseline mortality μ_0 of ants—helping is more profitable if μ_0 is small compared to ε_H; and (iii) the future added mortality rate (μ_J) of individuals that were injured and rescued.

The foraging behavior of *M. analis* seems to offer ideal conditions for rescue behavior to arise. Injury rates in combat ($\varepsilon_H = 0.17\%$ per day; for detailed calculations, "Quantification of model" section in Materials and Methods) seem to be large compared to the general mortality rate ($\mu_0 = 0.76\%$ per day), but injuries are rarely fatal (six observations of fatal injuries in 53 raids). Further, ants that recently lost a leg or had termites clinging onto their extremities are significantly hindered in their movement. This presumably makes returning

to the nest on their own costly in terms of energy and time needed, thereby prolonging exposure to potential predators and signaling a vulnerable state. These effects result in a high mortality risk of 32% for injured individuals if not helped (Fig. 2A). Carried injured individuals therefore benefit greatly from the rescue behavior, by reducing that risk to close to 0% (injured ants that were marked and rescued were observed again in subsequent raids in 38 of 40 cases and were never observed to die during the rescue process). Injured ants that are carried back recover from injuries in a short time, that is, parameter μ_J is close to ε_H, if we conservatively assume a second injury to be fatal. The fact that 21% of all ants carry some type of long-term injury in the raiding column (Fig. 4) substantiates the great value of helping injured nestmates, a conservative estimate because nonpermanent injuries are not included in this estimate. The value of rescue behavior is reflected in the sustainable colony size calculated by our model, which predicts a 28.7% larger colony size compared to colonies without this behavior.

DISCUSSION

This study shows the adaptive value of rescue behavior in a social predator specialized on a highly defensive prey—a behavior specifically focused on rescuing injured and handicapped individuals (remarkably also individuals that have permanent injuries in the form of lost extremities). Furthermore, by showing that this behavior is induced by pheromones, we support the hypothesis that the convergent evolution
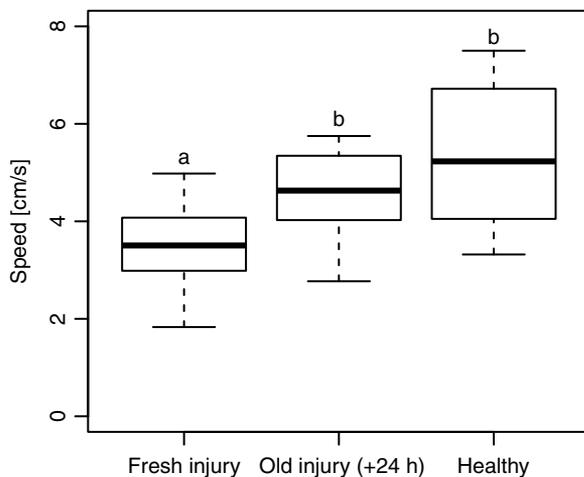
Fig. 3. Speed of injured ants at different times after injury. Box-and-whisker plot showing median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers), and significant differences (different letters) of the different running speeds 5 min after removing two legs (Fresh injury) and 24 hours later [Old injury (+24 hours)] and of healthy ants (Healthy) (Kruskal-Wallis rank sum test, followed by a Dunn's test with Bonferroni correction; *n* = 20 trials with five colonies). See also table S2 for detailed statistical results.

Fig. 4. Distribution of long-term injuries in different size classes of *M. analis*. Box-and-whisker plot showing median (horizontal line), interquartile range (box), distance from upper and lower quartiles times 1.5 interquartile range (whiskers), and significant differences (different letters) for the percentage of ants that lost an extremity in previous raids for majors, intermediates, and minors [analysis of variance (ANOVA), followed by Tukey post hoc test; *n* = 20]. See also table S3 for detailed statistical results.

of rescue behavior in different taxa has led to distinctive triggering mechanisms, such as chemical communication in insects or empathy in humans and possibly other mammals [(*23*, *24*); but see the study of Vasconcelos *et al.* (*21*) for other interpretations].

Rescuing injured individuals

Intermediates and minors carry injuries considerably more often than majors (Fig. 4). The division of labor at the hunting ground could explain this discrepancy. Whereas the smaller ants enter the termite galleries to hunt termites, the majors mostly focus on breaking up the protective soil layer over the hunting ground and carrying back the dead termites (*16*). Minors and intermediates are therefore far more exposed to injury risks.

A considerable number of carried ants did not seem to be injured (Fig. 1). Either the injuries were too small to be detected by the naked eye or these ants were truly unharmed. Most of the ants are picked up at the hunting ground after the fight, when the ants are preparing to leave. One possibility could be that the majors running over the hunting ground searching for leftover termites or injured individuals are less selective in what to carry. If they still encounter a minor, which might have lagged behind because it is inside the termite galleries, the major might just pick it up, thereby preventing it from falling behind even further while the column is already leaving.

The fact that experimentally injured ants are not picked up during the outward journey toward the termites seems to suggest that the behavior is context-specific (Fig. 5B). It seems unlikely for the ants to ever encounter this situation naturally. Furthermore, the rescue behavior would have to deviate from the natural one. If the helping ant would carry the injured individual back to the nest, it would expose itself to considerable predation risks by being forced to return alone while the rest of the column keeps marching to the termites. The other possibility would be to carry the injured ant all the way to the hunting ground only to have it carried back to the nest afterward. The different response necessitated by the helper ant in this situation and the very
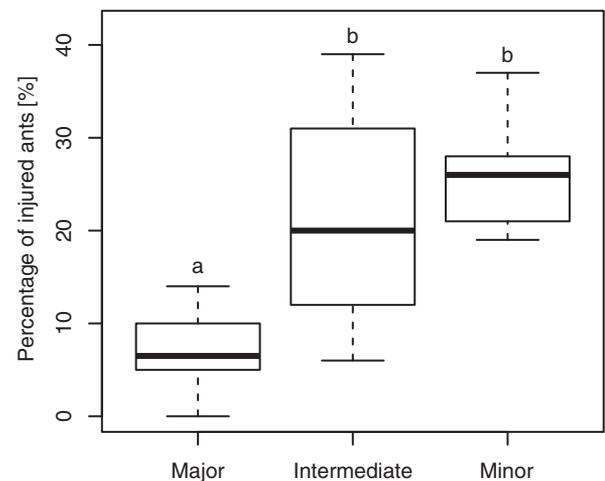
low injury risk on the outward journey most likely prevented the ants from developing a corresponding response.

We were able to show that this behavior is triggered through the chemical compounds DMDS and DMTS harbored in the mandibular gland reservoir; thus, this suite of compounds is a pheromone that seems to be specifically released when the individual is injured, in order to induce rescue behavior. The only other known species harboring this pheromone is the solitary hunting ponerine ant *Paltothyreus tarsatus*, in which the pheromone triggers digging behavior, most likely to rescue trapped nestmates (*25*). This species is in the same genus group as *Megaponera* (*8*), but being a solitary forager, *P. tarsatus* probably has not evolved the same kind of cooperative foraging-injury rescue behavior as in *M. analis*.

Cooperative self-defense has also been observed in *M. analis*, as a behavior in which nestmates scanned each other's legs and antennae and removed *Dorylus* sp. (driver ants) clinging to their extremities during encounters (*26*). The removal of these *Dorylus* ants seems to follow a mechanism similar to the removal of termite soldiers within the nest.

Evolution of helping the injured

We were able to assess the value of rescue behavior for injured individuals. Because assistance to individual ants is the main benefit of the rescue behavior, understanding the evolutionary benefit of this behavior for the colony as a whole is paramount. Ants that had lost an extremity do not immediately switch to a four- or five-legged locomotion mechanism but keep tripping over their phantom limbs. Ants that had termites clinging on them were even more severely handicapped in their movement (Fig. 1B). These ants were therefore unable to keep up with the returning column, fell behind, and thus became isolated from their nestmates. This retarded movement, on top of reduced dexterity, increased predation risk considerably (Fig. 2, A to E). Once termites clinging on their extremities were removed within the nest, they were able to fully perform again in future raids without any clear handicap. Ants that had lost an extremity had the benefit, after being carried back,
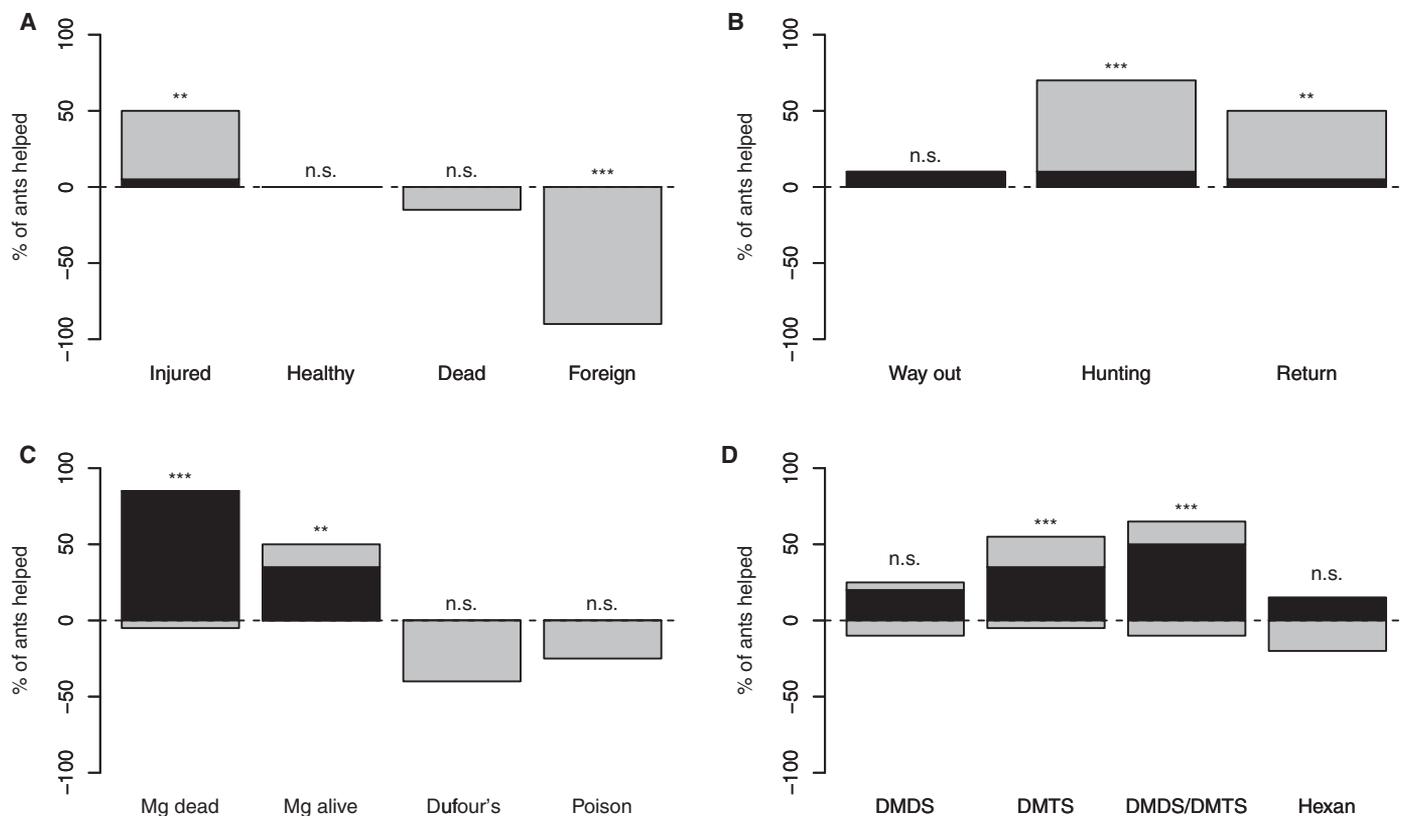
Fig. 5. Behavioral responses of helper ants toward different treatments of injured individuals or dummies. Positive values show clear attempts of help by picking up the ant and dropping it again (black) or carrying it back to the nest (gray). Negative values show behavior in which the ant was disposed of (dragged away from the raiding column or attacked) [Fisher's exact test for count data between neutral treatment (Healthy) and the other categories; $n = 20$]. (**A**) Response toward different injury states. (**B**) Response at different points of the raid (Way out: on the way toward the termites; Hunting: at the hunting ground; Return: on the return journey after the fight). (**C**) Response toward dummies (dead minors) treated with different glands (Mg dead: mandibular gland applied on a dummy; Mg alive: mandibular gland applied on a healthy/living ant; Dufours: Dufour's gland applied on a dummy; Poison: poison gland applied on a dummy). (**D**) Response toward dummies treated with different synthetic compounds (DMDS/DMTS: 50:50 mixture of DMDS and DMTS; Hexane: pure hexane as control). See also table S4 for detailed statistical results. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. n.s., not significant.

to recover from their injury in the safe environment of the nest, allowing them to get accustomed to a four- or five-legged locomotion. Thus, they reached running speeds similar to those of uninjured ants 24 hours later (Fig. 3). Because nearly all injured ants were observed in subsequent raids, we conclude that they carried no obvious long-term handicaps from their injuries and may fully participate again in colony tasks.

This type of rescue behavior, focused specifically on injured and handicapped individuals after hunting, is unique in social insects. Although the benefits seem obvious, there are several reasons as to why this has not yet been discovered in other species. First, because this behavior can only evolve in group-hunting species, where an injured ant can be detected by its nestmates, it excludes all solitary hunting species as potential candidates. Second, it is also essential that hunting occurs in isolated events, thus creating the risk for the injured ant to be separated from the group during the return journey. In a constantly occupied trail between ants and food source, the increased risk carried by an injured ant would be marginal, because it is constantly surrounded by nestmates warding off potential predators. In *M. analis*, the outward and return journeys are conducted as a discrete column with all ants marching together. The fact that the ants wait after the fight so that all ants may gather before returning to the nest (*11, 15*) exemplifies the importance of returning as a group. Third, the prey

species must be able to inflict a high amount of nonlethal injuries from which the ants can recover. With their large soldiers, termites fulfill this criterion as injury-inducing prey. Group foraging ant species that focus on leaf cutting, nectar, seeds, or scavenging are less likely to develop this rescue behavior of termite-raiding ants. Fourth, the benefit to the colony by the rescued ant has to outweigh the cost of help. In *M. analis*, the majors carry back termites and injured individuals; because minors sustain the most injuries (Fig. 4), the additional task for the majors to carry them back seems minimal from an energetic point of view (fig. S1A). Moreover, because on an average raid only 30% of the ants carry back prey (*11*), a large part of the workforce is available to help the injured individuals without decreasing the profits of the raid. Because the cost of helping an injured ant is therefore likely to be marginal in *M. analis*, it is thus ignored in our model. Last, the value of an individual for the colony plays an important role. This can be approximately quantified through the mean mortality rate in a colony. For a colony to be in equilibrium, the number of ants being born has to match the mortality rate, and in equilibrium, the population turnover is directly related to the life span of the individual. In *M. analis*, the population turnover is relatively low, with a birth rate of only 13 ants per day, demonstrating again the importance of rescuing the injured. Species with a very high turnover, such as army ants, seem likely to derive less

benefit from saving one injured ant, although this hypothesis remains to be tested. The specific biology of *M. analis* therefore provides the right circumstances where the benefit of saving the injured is especially large. We thus argue that this behavior evolved as part of an evolutionary arms race against termites, as a means of minimizing losses during raids and therefore foraging costs.

Rescue behavior has been previously observed in ants (*1*) but in very different contexts. Excavating trapped nestmates after a cave-in and rescuing an ant that fell in an antlion trap are both situations in which the individuals are confronted with an imminent danger, that is, suffocation or being eaten (*1*, *3*, *5*). This is not the case in our situation: Not only are the injured ants in many cases handicapped for life through the loss of extremities, but the immediate danger toward these ants is far less obvious. There is no direct threat to the injured ant but rather an abstract increased predation risk if these ants were to return alone. This study demonstrates that complex rescue behavior can evolve in very unique situations if the necessary drivers are present, even in species that are very likely unable to recognize the increased risks to which they are exposed to.

Outlook
Our observations offer a unique opportunity to experimentally study the evolutionary drivers leading to the emergence of rescue behavior in animals: Injury and predation rates can be manipulated, rescue behavior can easily be prevented, and critical variables and parameters can be measured. The Pan-African distribution of *M. analis* should also allow us to study the degree of fine-tuned adaptations to differing external selection pressures prevailing in different ecosystems and enable identification of the most important potential driving factors for evolution of this behavior. Our model also helps us to identify other candidate species in which this behavior might be found. Other ponerine genera, such as *Leptogenys*, also focus on hunting termites, with some of them hunting in groups (*27*); examining their raiding behavior in more detail could be promising. Slave-making ants could potentially also fulfill the criteria, if their prey can inflict a significant amount of nonlethal injuries.

MATERIALS AND METHODS
Experimental design
The study was conducted in a humid savannah woodland located in the Comoé National Park, northern Côte d'Ivoire (Ivory Coast), at the Comoé National Park Research Station (8°46′N, 3°47′W) (*28*). Observations throughout several days in April 2013 established that raiding activity was highest in the morning and afternoon hours between 0600 to 1100 and 1500 to 1900 local time, which corresponds to previous observations (*11*, *13*, *14*). Night raiding was also observed but was not included in this study. Experiments and observations were carried out in the field from 0700 to 1100 and 1500 to 1800 from April to September 2013, August to October 2014, and January to March and July to November 2015. *M. analis* is found throughout sub-Saharan Africa from 25°S to 12°N (*8*). We observed *M. analis* in a total of 52 different colonies for a total of 420 raids, in which the predominantly hunted termite species was *Pseudocanthotermes* sp. Living nests of *Macrotermes bellicosus*, which in other areas were often favored prey, could potentially cause a higher and more fatal injury rate due to their stronger soldiers; this species was absent in the vicinity of the study area. Colony size of 10 excavated colonies ranged between 900 and 2300 ants, a result comparable to previous studies in other regions

(*16*, *29*). Although *M. analis* is known to show monophasic allometry within its worker sizes (*16*, *30*) for statistical analysis and illustration, the workers were divided into majors (head width, >2.40 mm), minors (head width, <1.99 mm), and intermediates (head width, 2.40 to 1.99 mm), as proposed by Villet (*16*).

Quantification of carried ants
Experiments and observations were conducted in the field by waiting in front of a colony for a raid to be initiated and then following the raid column to the hunting ground. In total, we observed 420 raids in 52 different colonies. These 420 raids were used in various different experiments and observations.

To quantify the number of ants being carried back from the hunting ground, we counted the number of ants carrying a nestmate during the return journey shortly before arriving at the nest in a total of 53 raids. To classify the type of injuries in the carried ants (that is, with the following categories: lost limb, carried unharmed, and termite clinging), we retrieved the ants with forceps and investigated them. Injury inspections were conducted in 20 raiding columns of 20 different colonies for a total of 154 carried ants (Fig. 1A and fig. S1B).

To quantify the number of long-term injured ants participating in raids, we collected ants of all castes from 20 raids, each from a different colony, when raid columns were leaving the nest (that is, before any new fight could have taken place). In total, we collected 763 minors, 582 majors, and 502 intermediates (total $n = 1847$; Fig. 4).

Velocity and mortality
This experiment was conducted 20 times for each of the three categories (lost limb, termite clinging, and carried unharmed) in individual raids, with an additional control test of healthy individuals (Fig. 1, A and B). Individuals for the experiments were randomly selected from the pool of carried ants in a raid, with the control being a healthy ant walking unassisted in the returning raid column showing no sign of injury or handicap. Velocity was measured for the distance the ant followed the pheromone trail back to the nest. If a predator killed the ant during the return journey, the speed was calculated on the basis of the distance covered up to that incident. This allowed us to quantify the handicap and mortality risk that each injury posed during the return journey (Fig. 2, A to E). If the ant stopped moving during the return journey, most likely because of fatigue, the time was also stopped and the velocity was calculated up to that point.

Injury recovery
To analyze the potential recovery of ants that lost an extremity, we randomly cut off one leg on each side of a healthy ant (with scissors) and picked up during the return journey of the raid. The ant was then released on the return pheromone trail, and the covered distance in 60 s was measured. This experiment was repeated with the same individual 24 hours later and with healthy individuals as a control. These experiments were conducted with laboratory colonies, so that we could easily reproduce experimental parameters with regard to time between experiments and nest conditions. We used acrylic pens to mark individual ants, because previous observations showed no indication of any lasting disturbance to the ants with this marking method (fig. S2).

Ethogram of rescue behavior
Because there was no significant difference in the quantity of ants helped at the hunting ground or on the return journey (Fig. 5B; Fisher's exact

test, $P = 0.33$; $n = 20$), we carried out subsequent experiments (Fig. 5, A and C) during return journeys for easier reproducibility of trials. The experiments were repeated 20 times with at least five different colonies, with each return raid used for only one trial. For these experiments, an injured ant (or dummy) was placed at the front of the return column at least 1 m away from the hunting ground. All behavioral reactions by the nestmates were recorded until the entirety of the column passed the study subject. The behavioral reactions of the helping ants consisted of five categories: (i) Ignored: contact with the study subject was less than 2 s; (ii) Investigated: the study subject was antennated for more than 2 s; (iii) Picked up: the study subject was fully lifted from the ground; (iv) Carried back: the study subject was carried back for at least 20 cm toward the direction of the nest; (v) Carried away: the study subject was removed from the return column in a direction that was not the one back to the nest, that is, away from the column. For graphical illustration and statistical analysis, we summarized behaviors (iii) and (iv) as rescue behavior and (iii) in combination with (v) as disposing of the study subject.

Laboratory colonies

Ten colonies were excavated and placed in artificial nests in the field station laboratory (colony size, 1373 ± 520 ants), consisting of a 20 × 20 × 10–cm large nest made of polyvinyl chloride connected to a 1 × 1–m arena. For raids, this arena was connected by a 10-m-long corridor to a second arena (1 × 1 m). The ground was covered with earth from the surrounding area. In the second arena, *M. bellicosus* termites were placed, which were collected from the surrounding area with a pot filled with dry grass. These termites were found by scouts and triggered raiding behavior on which we performed the injury recovery experiments. For further details on laboratory keeping, see Yusuf *et al.* (*29*).

Pheromone and stridulatory communication

To inhibit stridulation, we coated the stridulatory organ, located between the first and second tergites (*22*), with black acrylic paint. After the paint dried for 2 min, the experiment was conducted. To confirm that stridulation was truly inhibited, we triggered normal stridulation behavior by exposing the ant to CO_2, as previously described by Hölldobler *et al.* (*22*). During this process, the sound was recorded with an external microphone (Speedlink SL-8703-BK, Jöllenbeck GmbH). To visualize the sound, a sonogram was created with the digital audio editor Audacity version 2.0.5.0 (fig. S1A).

For the pheromone experiments, we dissected a gland and placed it on a glass surface, then pulled the thorax of the study subject three times over the burst gland reservoir (for smaller mandibular glands, two glands were used per experiment). For the experiments with synthetic chemicals, we first diluted the substance in hexane until we reached a concentration of 90 ng/ml. Subsequently, two drops (roughly 9 ng of the substance) were applied on a glass surface. The selected concentrations were similar to the quantities found in a mandibular gland [14 ng of DMDS and 5 ng of DMTS per gland in a major worker according to Longhurst *et al.* (*17*)] and a comparison of the mass spectrometer of the gland reservoir with our solution. After 30 s, most of the hexane evaporated and the thorax of the dummy was pulled over the glass surface three times.

Chemical analysis

Foraging *M. analis* workers were collected from various colonies at the Comoé National Park (Côte d'Ivoire). The workers were then transported alive to the University of Würzburg (Germany) and killed with CO_2 before excision of the mandibular gland reservoirs. The caput and the mandibles, including the mandibular gland, of 20 ants were then soaked in 1 ml of pure pentane for 2 hours (two caputs and six mandibular glands, respectively). These extracts were evaporated to a residue of approximately 100 μl. We used 1 μl extracts for GC-MS analyses, which were carried out on a gas chromatograph (6890) coupled to a mass selective detector (5975) from Agilent Technologies. The GC was equipped with a DB-5 capillary column (0.25 mm inside diameter × 30 m; film thickness, 0.25 μm; J&W Scientific). Helium was used as a carrier gas, with a constant flow of 1 ml/min. A temperature program from 60° to 300°C with 5°C/min and finally 10 min at 300°C was used, with data collection starting 2 min after injection. The mass spectra were recorded in the electron ionization mode, with an ionization voltage of 70 eV and a source temperature of 230°C.

The software ChemStation (Agilent Technologies) for Windows was used for data acquisition. Identification of the components was accomplished by comparison with purchased chemicals and the use of a commercial MS database (NIST 4.0). Because of the very small quantities of DMDS and DMTS within the extracts, we used diagnostic ions and the retention time to confirm the identification.

Rescue behavior model

We designed an equilibrium model to quantify the possible benefits of rescue behavior to the colony. Benefit was expressed as the proportional increase in equilibrium worker number of a colony with rescue behavior compared to a colony that would not show this behavior. For the sake of argument, we chose a very simple model that does not account for all the mechanisms that truly regulate worker numbers in ant colonies.

We assume that the worker dynamics of a colony without rescue behavior is described by equation

$$\frac{dH}{dt} = b - (\varepsilon_H + \mu_0)H \tag{1a}$$

where H is the number of noninjured (healthy) workers, b is the rate at which new workers are added to the colony, ε_H is the rate at which workers are involved in injuring interactions with termites, and μ_0 is the base mortality rate of workers.

For this colony, colony size (worker number) will settle into equilibrium

$$\hat{H} = \frac{b}{\varepsilon_H + \mu_0} \tag{1b}$$

In addition, a colony that manages to rescue a fraction f ($0 \le f \le 1$) of the workers injured in action will build a "pool" J of workers that were injured in previous raids but were rescued; conservatively, we do not separate between injured ants that may ultimately recover (and would thus return to pool H) and workers that carry permanent damages, such as a lost extremity. The dynamics of injured ants is described as

$$\frac{dJ}{dt} = f\varepsilon_H H - (\mu_J + \mu_0)J \tag{2a}$$

where f ($0 \le f \le 1$) is the proportion of ants injured in combats that survive and μ_J is the added (future) mortality rate of injured compared to

to noninjured workers. For simplicity and on the basis of empirical observation, we conservatively assume that a second injury sustained in another raid would always be fatal. The equilibrium number of injured ants in a colony is thus

$$\hat{J} = \hat{H} \frac{f\varepsilon_H}{\mu_J + \mu_0} \quad (2b)$$

The relative size of colonies with rescue behavior compared to a colony not showing this behavior, that is, a total loss of injured individuals ($f = 0$), is thus defined by

$$\frac{\hat{H} + \hat{J}}{\hat{H}} = 1 + \frac{f\varepsilon_H}{\mu_J + \mu_0} \quad (3)$$

Quantification of model

The observed survivability of an injured ant not receiving help is 68% (Fig. 2A). Thus, $f = 0.68$ characterizes hypothetical colonies without rescue behavior, whereas in colonies where the behavior is present, $f = 1$, because all rescued ants were observed in later raids. All other parameters stay the same in both cases and were calculated as follows. Because we can only quantify the injury ratio in the colony for ants that lost an extremity, our value ε_H was defined as the percentage of lost limb injuries per raid (0.21·3) per day (3) divided by the ratio of healthy ants in a colony (0.79·1373, $n = 10$ excavated colonies); therefore, $\varepsilon_H = 0.0017$. We conservatively argue that the added mortality of a previously injured ant is the probability of getting injured again; therefore, $\mu_J = \varepsilon_H$, in our scenario. We estimated the birth rate b of the colony by observing the callow worker population of excavated nests until they were fully sclerotized (106 ± 30 callow workers per excavated colony, $n = 5$). Sclerotization time was calculated to be 8 days on average ($n = 5$), leading us to an estimate of 13.3 ± 3.8 ants born per day. In Eq. 1b, we were able to calculate $\mu_0 = 0.0076$. To test the precision of the parameters estimated from empirically observed data, we compared the empirical ratio of injured ants in the colony (0.21; Fig. 4) to our model prediction from Eq. 2b: 0.21. The good agreement of predicted and empirical values allowed us to reliably calculate the benefit of the rescue behavior by comparing the calculated colony size ($H + J$) of a colony with rescue behavior to one without. Our results indicate that the helping behavior results in a 6.0% larger colony size if we just consider the benefit for the 21% of carried ants that lost an extremity (Fig. 1A). If extrapolated for all injuries, the benefit of the rescue behavior can be estimated to be 28.7%.

Statistical analysis

For statistical analysis and graphical illustration, we used the statistical software R version 3.1.2 (31) with the user interface RStudio version 0.98.501. We tested for deviations from the normal distribution with the Shapiro-Wilk test ($P > 0.05$). A Bartlett test was used to verify homoscedasticity ($P > 0.05$). If data were normally distributed and homoscedastic, an ANOVA was used to compare the significance of the results with a Tukey post hoc test for post hoc analysis. If this was not the case, a Kruskal-Wallis rank sum test was used, followed by a Dunn's test with Bonferroni correction. To analyze the ethogram data, a Fisher's exact test with Bonferroni correction was used with a no-help control (0 of 20 helped) compared to our treatments. Median values mentioned in the text are followed by a median absolute deviation.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/3/4/e1602187/DC1

fig. S1. Illustration of a helping ant and different injury types, as shown in Fig. 1.
fig. S2. Effect of stridulation on rescue behavior.
table S1. Statistical differences in injury-type frequency (Fig. 1A) and speed (Fig. 1B) in injured *M. analis* ants (Kruskal-Wallis test, followed by pairwise comparisons with Bonferroni-corrected Dunn's test; $n = 20$ per test).
table S2. Statistical differences in running speed of individuals with different stages of injury, as shown in Fig. 3 (Kruskal-Wallis test, followed by Dunn's test with Bonferroni correction; $n = 20$ per test).
table S3. Statistical differences in long-term injuries in the different castes, as shown in Fig. 4 (ANOVA test, followed by Tukey post hoc test; $n = 20$ per test).
table S4. Statistical differences in significance of rescue behavior compared to behavior of healthy individuals, as shown in Fig. 4 (A to D) and fig. S2 [Fisher's exact tests for count data between treatment healthy (no help) and the other categories with Bonferroni correction].
movie S1. An injured ant receiving help by its nestmates.

REFERENCES AND NOTES

1. K. L. Hollis, E. Nowbahari, Toward a behavioral ecology of rescue behavior. *Evol. Psychol.* **11**, 647–664 (2013).
2. E. Nowbahari, A. Scohier, J.-L. Durand, K. L. Hollis, Ants, *Cataglyphis cursor*, use precisely directed rescue behavior to free entrapped relatives. *PLOS ONE* **4**, e6573 (2009).
3. K. L. Hollis, E. Nowbahari, A comparative analysis of precision rescue behaviour in sand-dwelling ants. *Anim. Behav.* **85**, 537–544 (2013).
4. K. L. Hollis, F. A. Harrsch, E. Nowbahari, Ants vs. antlions: An insect model for studying the role of learned and hard-wired behavior in coevolution. *Learn Motiv.* **50**, 68–82 (2015).
5. W. Hangartner, Carbon dioxide, a releaser for digging behavior in *Solenopsis geminata* (Hymenoptera: Formicidae). *Psyche* **76**, 58–67 (1969).
6. K. Taylor, A. Visvader, E. Nowbahari, K. L. Hollis, Precision rescue behavior in North American ants. *Evol. Psychol.* **11**, 665–677 (2013).
7. B. Hölldobler, E. O. Wilson, *The Ants* (Belknap Press of Harvard Univ. Press, 1990).
8. C. A. Schmidt, S. O. Shattuck, The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of Ponerine ecology and behavior. *Zootaxa* **3817**, 1–242 (2014).
9. J. Levieux, Note préliminaire sur les colonnes de chasse de *Megaponera fœtens* F. (Hyménoptère Formicidæ). *Insect. Soc.* **13**, 117–126 (1966).
10. C. Longhurst, R. Baker, P. E. Howse, Termite predation by *Megaponera foetens* (FAB.) (Hymenoptera: Formicidae). *J. Chem. Ecol.* **5**, 703–719 (1979).
11. J. Bayliss, A. Fielding, Termitophagous foraging by *Pachycondyla analis* (Formicidae, Ponerinae) in a Tanzanian coastal dry forest. *Sociobiology* **39**, 103–122 (2002).
12. M. G. Lepage, Étude de la prédation de *Megaponera foetens* (F.) sur les populations récoltantes de Macrotermitinae dans un écosystème semi-aride (Kajiado-Kenya). *Insect. Soc.* **28**, 247–262 (1981).
13. A. A. Yusuf, I. Gordon, R. M. Crewe, C. W. W. Pirk, Prey choice and raiding behaviour of the Ponerine ant *Pachycondyla analis* (Hymenoptera: Formicidae). *J. Nat. Hist.* **48**, 345–358 (2014).
14. C. Longhurst, P. E. Howse, Foraging, recruitment and emigration in *Megaponera foetens* (Fab.) (Hymenoptera: Formicidae) from the Nigerian Guinea Savanna. *Insect. Soc.* **26**, 204–215 (1979).
15. B. Corbara, A. Dejean, Adaptive behavioral flexibility of the ant *Pachycondyla analis* (=*Megaponera foetens*) (Formicidae : Ponerinae) during prey capture. *Sociobiology* **36**, 465–483 (2000).
16. M. H. Villet, Division of labour in the Matabele ant *Megaponera foetens* (Fabr.) (Hymenoptera Formicidae). *Ethol. Ecol. Evol.* **2**, 397–417 (1990).
17. C. Longhurst, R. A. Johnson, T. G. Wood, Predation by *Megaponera foetens* (Fabr.) (Hymenoptera: Formicidae) on termites in Nigerian southern Guinea savanna. *Oecologia* **32**, 101–107 (1978).
18. G. D. Prestwich, Defense mechanisms of termites. *Annu. Rev. Entomol.* **29**, 201–232 (1984).
19. W. Sheppe, Invertebrate predation on termites of the African savanna. *Insect. Soc.* **17**, 205–218 (1970).
20. L. Burgeon, Une organisation sanitaire chez les fourmis Megaponera. *Rev. Zool. Bot. Afr.* **16**, 94–95 (1929).
21. M. Vasconcelos, K. Hollis, E. Nowbahari, A. Kacelnik, Pro-sociality without empathy. *Biol. Lett.* **8**, 910–912 (2012).
22. B. Hölldobler, U. Braun, W. Gronenberg, W. H. Kirchner, C. Peeters, Trail communication in the ant *Megaponera foetens* (Fabr.) (Formicidae, Ponerinae). *J. Insect. Physiol.* **40**, 585–593 (1994).
23. I. B.-A. Bartal, J. Decety, P. Mason, Empathy and pro-social behavior in rats. *Science* **334**, 1427–1430 (2011).

24. J. Decety, I. B.-A. Bartal, F. Uzefovsky, A. Knafo-Noam, Empathy as a driver of prosocial behaviour: Highly conserved neurobehavioural mechanisms across species. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **371**, 20150077 (2016).

25. R. M. Crewe, D. J. C. Fletcher, Ponerine ant secretions: The mandibular gland secretion of *Paltothyreus tarsatus* Fabr. *J. Entomol. Soc. South. Afr.* **37**, 291–298 (1974).

26. J. Beck, B. K. Kunz, Cooperative self-defence: Matabele ants (*Pachycondyla analis*) against African driver ants (*Dorylus* sp.; Hymenoptera: Formicidae). *Myrmecol. News* **10**, 27–28 (2007).

27. K. N. Ganeshaiah, T. Veena, Topology of the foraging trails of *Leptogenys processionalis*—Why are they branched? *Behav. Ecol. Sociobiol.* **29**, 263–270 (1991).

28. P. Koffi, Examples of protected areas by ecosystem type: Comoé National Park, in *Biodiversity Atlas of West Africa, Volume 3: Côte d'Ivoire*, S. Konaté, D. Kampmann, Eds. (BIOTA, 2010).

29. A. A. Yusuf, R. M. Crewe, C. W. W. Pirk, An effective method for maintaining the African termite-raiding ant *Pachycondyla analis* in the laboratory. *Afr. Entomol.* **21**, 132–136 (2013).

30. R. M. Crewe, C. P. Peeters, M. Villet, Frequency distribution of worker sizes in *Megaponera foetens* (Fabricius). *S. Afr. J. Zool.* **19**, 247–248 (1984).

31. R Core Team (R Foundation for Statistical Computing, 2013).

Citation: E. T. Frank, T. Schmitt, T. Hovestadt, O. Mitesser, J. Stiegler, K. E. Linsenmair, Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*. *Sci. Adv.* **3**, e1602187 (2017).

Science Advances

Saving the injured: Rescue behavior in the termite-hunting ant *Megaponera analis*

Erik Thomas Frank, Thomas Schmitt, Thomas Hovestadt, Oliver Mitesser, Jonas Stiegler and Karl Eduard Linsenmair

ARTICLE TOOLS	http://advances.sciencemag.org/content/3/4/e1602187
SUPPLEMENTARY MATERIALS	http://advances.sciencemag.org/content/suppl/2017/04/10/3.4.e1602187.DC1
REFERENCES	This article cites 28 articles, 1 of which you can access for free http://advances.sciencemag.org/content/3/4/e1602187#BIBL
PERMISSIONS	http://www.sciencemag.org/help/reprints-and-permissions

Use of this article is subject to the Terms of Service